

PATENT ABSTRACTS OF JAPAN

(11)Publication number : 09-157266

(43)Date of publication of application : 17.06.1997

(51)Int.Cl.

C07D303/36
A61K 31/335
A61K 31/335
C12P 17/02
/(C12P 17/02
C12R 1:01)

(21)Application number : 07-315542

(71)Applicant : MICROBIAL CHEM RES FOUND

(22)Date of filing : 04.12.1995

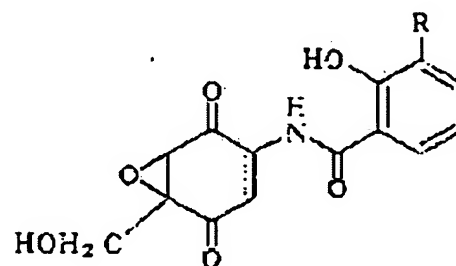
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(54) NEW ANTIBIOTIC EPOXYNOMICIN A AND B AND THEIR PRODUCTION

(57)Abstract:

PROBLEM TO BE SOLVED: To obtain new antibiotics, epoxynomicin A and B, exhibiting antitumor activities and antibacterial activities against gram-positive bacteria including methicillin-resistant bacteria.

SOLUTION: Antibiotics, epoxynomicin A and B, of the formula (R is chlorine and H in the epoxynomicin A and B, respectively). The epoxynomicin A has the following physicochemical properties. Appearance and properties: pale yellowish powder, weakly acidic substance; melting point: 168-173°C (decomposition); specific rotation: $[\alpha]_{D25} +44.6^\circ$ (C 0.51, methanol); R_f value of TLC: 0.28; high resolution mass spectrum: experimental value: 332.0136 (M-H), calculated value: 332.0118; molecular



formula: C₁₄H₁₀NO₆Cl; UV light spectrum: λ_{max} nm (ϵ) 236 (sh,8900), 255 (sh,5900), 325 (sh, 8000), 370 (sh, 2700) (methanol solution). The compound of the formula is obtained by culturing an epoxynomicin A and B-producing fungus [Amycolatopsis sp. MK 299-95F4 (FERM P-15243)] belonging to the genus Amycolatopsis in a nutritive medium under an aerobic condition.

LEGAL STATUS

[Date of request for examination]

[Date of sending the examiner's decision of rejection]

[Kind of final disposal of application other than the examiner's decision of rejection or application converted registration]

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[Patent number]

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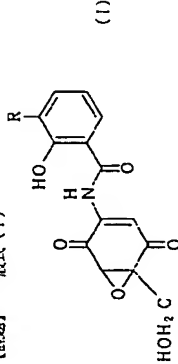
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(19)日本国特許庁 (J P) (12) 公開特許公報 (A)

(11)特許出願公開番号
特開平9-157266
(43)公開日 平成9年(1997)6月17日

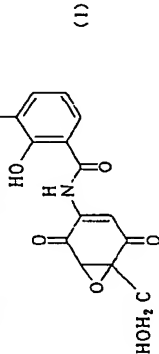
(51)Int.Cl. ⁴	識別記号	庁内整理番号	F I	技術表示箇所
C 0 7 D 3 0 3 / 3 6	A D U		C 0 7 D 3 0 3 / 3 6	
A 6 1 K 3 1 / 3 5	A D Z		A 6 1 K 3 1 / 3 5	A D U
C 1 2 P 1 7 / 0 2			C 1 2 P 1 7 / 0 2	A D Z
// (C 1 2 P 1 7 / 0 2				
(21)出願番号	特開平7-315542	(71)出願人	000173013	請求項の最 5 O L (全 14 頁) 最終頁に続く
(22)公開日	平成 7 年 (1995) 12 月 4 日	財団法人微生物化学研究会		
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(式中、RはエポキシノマイシンAでは塩基原子を示し、エポキシノマイシンBでは水素原子を示す) で表わされるエポキシノマイシンAおよびエポキシノマイシンBが新規抗菌物質としてアミコラプトシス sp. WX299-95F 4株の培養により得られた。エポキシノマイシンAおよびB、あるいはそれらの塩は各種の細菌に対する抗菌活性と抗腫瘍活性とを有する抗菌物質である。

【特許請求の範囲】

【請求項1】 次の一般式 (1) :



(式中、RはエポキシノマイシンAでは塩基原子を示し、またエポキシノマイシンBでは水素原子を示す) で表わされる化合物である抗菌物質エポキシノマイシンAおよびエポキシノマイシンB、またはそれらの塩。

【請求項2】 アミコラプトシス菌に属する、請求項1に記載のエポキシノマイシンAおよびBの生産菌を培養し、培養物からエポキシノマイシンAおよびBを採取することを特徴とする、抗菌物質エポキシノマイシンAおよび(または)エポキシノマイシンBの製造法。

【請求項3】 抗菌物質エポキシノマイシンAおよび(または)エポキシノマイシンB、またはそれらの塩を有効成分とする抗腫瘍剤。

【請求項4】 抗菌物質エポキシノマイシンAおよび(または)エポキシノマイシンB、またはそれらの塩を有効成分とする抗腫瘍剤。

【請求項5】 抗菌物質エポキシノマイシンAおよびエポキシノマイシンBを生産する特性を持つアミコラプトシス sp. WX299-95F4株。

【発明の詳細な説明】

【0001】

【発明の属する技術分野】本発明は、抗菌活性及び抗菌活性または抗腫瘍活性を示す新規抗菌物質エポキシノマイシンAおよび(または)エポキシノマイシンB、あるいはこれらに属する、またエポキシノマイシンAおよび(または)エポキシノマイシンBの製造法に関する。さらに本発明は、エポキシノマイシンAおよび(または)エポキシノマイシンBまたはそれらの塩を有効成分とする抗腫瘍剤及び抗腫瘍剤に関する。また、本発明は新規抗菌物質エポキシノマイシンAおよびBを生産する特性を持つ新規な微生物としてのアミコラプトシス sp. WX299-95F4株を含む。

【0002】

【従来の技術】種々な多数の抗菌性物質が知られており、また種々な多数の抗腫瘍性物質が知られている。

【0003】

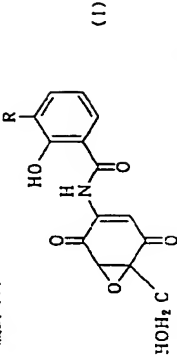
【発明が解決しようとする課題】細菌感染症の化学療法において、多剤耐性菌の出現は重大な問題である。従来知られているまたは使用されている既知の抗菌性化合物

とは、異なる化学構造を有し且つ優れた抗菌活性を示す新しい化合物の発見または創製をすることは常に望まれており、そのための研究が行われている。また抗腫瘍性物質は、一般に強い毒性を有するものが多く、その抗腫瘍剤としての使用に当たって大きな制約となっている。そこで、毒性が低く且つ新規な化学構造を有する抗腫瘍性物質を発見または創製することが常に望まれており、そのための研究が行われている。

【0004】

【課題を解決するための手段】本発明者らは、上記の要望に応えることができる抗菌活性及び抗腫瘍活性を持つ新規な抗菌物質を提供することを目的に、従来より有用な抗菌物質の開発と実用化の研究を促進してきた。その結果、土壌試料から新規な微生物としてアミコラプトシス菌に属する菌株を分離することに成功し、またこの菌株が新しい構造骨格を有する複数の抗菌物質を生産していることを見出した。これら新規抗菌物質2種を単離することに成功し、それぞれにエポキシノマイシンAおよびエポキシノマイシンBと命名した。更に、これら2種の抗菌物質がメチリン耐性菌(メチリン耐性菌等)をふくむグラム陽性の細菌に抗菌活性を示し、また癌細胞の増殖に対して抑制活性を示すことを見出した。

【0005】すなわち、第1の本発明においては、次の一般式 (1) :



(式中、RはエポキシノマイシンAでは塩基原子を示し、またエポキシノマイシンBでは水素原子を示す) で表わされる化合物であるエポキシノマイシンAおよびエポキシノマイシンB、あるいはこれらに属する、またエポキシノマイシンAおよびBは、第4級アミン性物質であり、それらの塩としては、第4級アミン性物質などの有機塩基との塩、あるいは各種金属との塩、例えばナトリウムのようなアルカリ金属との塩があり、これらの塩も上記の抗菌活性と抗腫瘍活性を有する。

【0007】次に、抗菌物質エポキシノマイシンAおよびBの理化学的性質を記載する。

(1) エポキシノマイシンAの理化学的性質

A) 外觀及び性質：淡黄色粉末、結晶性物質

B) 融点：168-173℃ (分解)

C) 比旋光度：(α)_D²⁵+44.6° (c 0.51, メタノール)

D) T L C の R f 値：0.28

シリカゲル (Art. 105715, メルク社製) の薄層クロマト

グラフィーで展開溶媒としてクロロホルム-メタノール

E) マススペクトル (m/z) : 324, 326 (M+H)・

322, 324 (M-H)・

F) 高分解能マススペクトル: 実測値 322.0136 (M-H)・

計算値 322.0118

G) 分子式: $C_{14}H_{16}NO_5$ C 1 H 1 紫外線吸収スペ

クトル: $\nu_{max}(cm^{-1})$ 3450, 1710, 1670, 1600, 1520, 1460, 1

340, 1230

J) ^{13}C -NMR スペクトル (CD_3OD/TMS): 添

付図面の図5に示す。

K) 1H -NMR スペクトル (CD_3OD/TMS): 添

付図面の図6に示す。

【0008】(2) エポキシノマイシンBの理化学的性

状

A) 外観及び性質: 淡黄色粉末, 弱酸性物質

B) 融点: $178-184^{\circ}C$ (分解)

C) 比旋光度: $(\alpha)_D^{25} +32.2^{\circ}$ (c 0.51, メタノ

ール)

D) TLCのRf値: 0.52

シリカゲル (Art. 105715, メルク社製) の薄層クロマト

グラフィーで展開溶媒としてクロロホルム-メタノール

(10:1) で展開して測定した場合

E) マススペクトル (m/z) : 289 (M)・

288 (M-H)・

F) 高分解能マススペクトル: 実測値 290.0656 (M+H)・

計算値 290.0664

G) 分子式: $C_{14}H_{16}NO_5$

H) 紫外線吸収スペクトル:

(1) メタノール溶液中で測定したUV吸収スペクトル

を添付図面の図7に示す。主なピークは次のとおりであ

る。

$\lambda_{max} nm (e)$ 237(6100), 253(sh, 5400), 326(6300)

(11) 0.01N NaOH-メタノール溶液中で測定した吸収

スペクトルは添付図面の図8に示す。主なピークは次の

とおりである。

$\lambda_{max} nm (e)$ 235(9100), 259(sh, 4000), 324(5800),

370(sh, 3400)

(11) 0.01N HCl-メタノール溶液中で測定したUVス

ペクトルは添付図面の図9に示す。主なピークは次の

とおりである。

$\lambda_{max} nm (e)$ 252(5700), 327(6500)

I) 紫外線吸収スペクトル (KBr錠剤法): 添付図面

【0011】

トン登天岩地で倍収希釈法により測定した。

【0011】

(表1)

試 験 値	最低有効阻止濃度 ($\mu g/ml$)	
	エポキシキノ	マイシンB
スタヒロコッカス・アクレウス FIM 209P	12.5	12.5
スタヒロコッカス・アクレウス・スミス	12.5	12.5
スタヒロコッカス・アクレウス MS 9610	50	25
スタヒロコッカス・アクレウス MDSA No.5	25	25
スタヒロコッカス・アクレウス MS 16526	25	25
スタヒロコッカス・アクレウス TY-04282	50	25
マイクロコッカス・ルテウス FIM 16	12.5	25
マイクロコッカス・ルテウス IF0 3303	3.12	0.25
バシルス・アンズラシス	25	12.5
バシルス・サブチリス NRRL B-558	50	12.5
バシルス・セレウス ATCC 10702	25	12.5
コリネバクテリウム・ボビス 1810	50	50
エシェリヒア・コリ NIHJ	100	50
エシェリヒア・コリ BE 1121	50	50
エシェリヒア・コリ BB 1186	50	50
シゲラ・デイスンテリエ JS 11910	50	50
シゲラモナス・エルギノサ A 3	>50	>50
バストレラ・ペンシダ sp. 6395	12.5	12.5
バストレラ・ペンシダ sp. 0356	12.5	12.5
バストレラ・ペンシダ p-3047	3.2	12.5

【0012】B) 癌細胞増殖抑制活性

各種の癌細胞を用いて癌細胞の増殖を50%抑制するエポ

キシノマイシンAおよびエポキシノマイシンBの濃

度 (IC_{50} 値) を、MTT法 ([Journal of Immunologic

【表2】)

供 試 癌 胞	$IC_{50} (\mu g/ml)$	
	エポキシキノ	マイシンB
マウス白血球 L1210	2.64	16.3
マウス IMCカルシノーマ	9.67	17.9
マウスザルコーマ S180	7.67	
マウス黒色腫 B16-BL6	7.97	

【0014】表1の結果から明らかなように、本発明に

よる抗生物質エポキシキノマイシンAおよびBは、各種

の細胞に対して抗癌活性を有するから抗癌剤として有用

である。また、表2の結果から明らかなように、エポキ

キシノマイシンAおよびBは各種の癌細胞の増殖を抑制

する抗癌増殖活性または抗癌活性を有するから抗癌増殖

たは抗癌剤として有用である。

【0015】さらに第2の本発明によれば、アミノコラト
ブシス属に属する、前記一般式(1)のエポキシキノ
マイシンAおよびBの生産を培養増地に特徴し、培養
効率がエポキシノマイシンAおよび(または)エポキ
シノマイシンBを生成することを特徴とする、抗生物
質エポキシキノマイシンAおよび(または)エポキシ
ノマイシンBの製造法が提供される。

【0016】第2の本発明の方法で使用できるエポキシキノマイシンAおよびBの生産物の一例としては、アミコラゴベアズ、株、WX299-95F4株がある。この菌株は平成6年10月、微生物化学研究所において、宮城県仙台市の土壌より分離された放線菌で、WX299-95F4の菌株番号が付された微生物である。

【0017】このW299-95F4株の樹学的性状を次に記載する。

1. 形態

寄生菌糸はよく分枝し、ジグザグ状を呈する。また分枝が認められる。菌糸糸は直状あるいは不規則な曲状で、円筒形〜梨円形の断片または胞子殻の構造に分断する。その殻面は平滑であり、大きさは約 $0.4 \sim 0.6 \times 1.1 \sim 1.6$ ミクロンである。輪生枝、胞子糸のうろ及び運動性菌糸は認められない。

【0018】2. 各型各地における生育状態の記載について〔 〕内に示す標準は、コンテナカラー・コーポレーション・オブ・アメリカのカラ・ハーモニー・マニュアル (Container Corporation of America の color harmony manual) を用いた。

(II) シュクロース・硝酸塩寒天培地 (27℃培養)
無色の発育上に、白の気団糸をうっすらと増生し、溶解性色素は認められない。

(2) グルコース・アスパラギン寒天培地 (27℃培養)
うす黄 (2 ea, Lt Wheat ~ 2 gc, Bamboo) の発育上、
白の気菌系を発生し、溶解性色素は菌を帯びる。

(3) グリセリン・アスパラギン寒天培地 (ISP-培地 5、27℃培養)

質を抽出し、上記と同様に単離精製することができる。
かくして、前記した特性を有する新規抗生物質エボキシ
キノマイシンAおよびBが得られる。

(1) 一般に、

【0027】さらに、好適な実施形態では、図1Aで取除されるエポキシキ/マイシンAおよび（または）エポキシキ/マイシンBまたはそれらの製薬学的に許容される塩を有効成分とする抗悪阻が提供される。

【0033】また、第4の本発明においては、一般式
(1)で表されるエポキシキノマイシンAおよび（または）エポキシキノマイシンBまたはそれらの製薬学的に許容できる塩を有効成分とする抗腫瘍剤が提供される。

【0034】この抗菌剤または抗腫瘍剤においては、有効成分としてのエポキシキノマイシンAおよび（または）Bあるいはその塩は製薬学的に許容できる常用の固形剤または液体剤、例えばエタノール、水、でん粉等と混合されている形の組成物であることができる。

【0035】また、第5の本発明では、新規な微生物として、前記の一般式(1)のエポキシノマイシンAおよびBを生産する特性を持つアミコラトブシス sp. M2-99-95F4 株が提供される。

【0036】

【発明の実施の形態】次に実施例により本発明を更に詳細に説明するが、本発明は下記の実施例に限定されるものではない。

【0037】実施例1 抗生物質エポキシノマイシン
AおよびBの製造

グリセリン 0.5%、シュウケロース 2%、大豆粉 1%、
 酵母粉 1%、コンスタープ・リガー 0.5%、
 塩化ナトリウム 0.001% を含む液体培地 (pH7.0-7.5)
 箱) を三角フラスコ (500ml 容) に 110ml ずつ分注し、常
 法により 120℃ で 20 分滅菌した。これらの培地に、寒天
 斜面培地に播菌した *アミココリナシス* sp. MW299-95F4
 株 (FEDU P-15243) を接種し、その後 30℃ で 5 日間回転
 振とう培養した。これにより、菌田培養を得た。

【0038】グリセリン 2%、デキストリン 2%、
 バンクソール 1%、精製酢酸エキス 0.3%、硫酸
 アンモニウム 0.0%、炭酸塩化物 0.2%、シリコーン
 オイル 1滴を含む炭酸塩化物 (pH 7.4) に調整した魚肝油
 スコ (500mg/3%) に 10ml ずつを注し、常法により 120℃
 で 20分滅菌した。その後、これを均等に、上庄田牧場
 牧場それぞれ 2g ずつ接種し、27℃で 4日間培養した。

【0039】このようにして得られた培養液を濾過して固体を分離した。培養液2.55リットルは、6 N-HClにより調製した魚肝油酢酸ナトリウム溶液で抽出し、酢酸ブチル脂を無水硫酸マグネシウムにより乾燥した。酢酸ブチル脂を破滅下で濃縮乾固し、残渣をメタノール(5ml)に溶かし、ヘキサメチルアミン(2回洗浄、1回5ml)で洗った。

ル目を減圧下で濃縮乾固した。

【0004】得られた残渣をクロロホルム-メタノール水(50:10; 40:10m/v)で処理し、下層を注下して乾燥油状物を、着色油状物(0.515 g)が得られた。この乾燥油物を、シリカゲルカラムクロマトグラフィー(Kiesel gel 60, メルク社製 50μ)に付き、トルエン-アセトン混合溶媒(10:1, 7:1, 5:1, 3:1, 2:1)で順次抽出した。得られた活性面成分を条件付のシリカゲルで抽出したクロマトフーリーに付き、トルエン-アセトン混合溶媒(50:1, 20:1, 10:1, 7:1)で抽出物が124mg得られた。この混合物の35mgをシリカゲルTLC(展開溶媒:クロロホルム-メタノール, 20:1)にかけて分離精製した。

【0004.1】エポキシノマイシンAが熔点 168~173℃(分解)の淡黄色粉末とし280gの収量で得られ、またエポキシノマイシンBが熔点 178~184℃(分解)の淡黄色粉末とし10mgの収量で得られた。

【図面の簡単な説明】
【図１】エポキシ/マイシンＡのメタノール溶液中の紫外線吸収スペクトルである。

【図2】エポキシキノマイシンAの0.01N NaOH—メタノール溶液中の紫外線吸収スペクトルである。

【図3】エポキシノマイシンAの0.01N HCl-メタノール溶液中の紫外線吸収スペクトルである。

【図4】エポキシノマイシンAのKBr錠剤法で測定した赤外線吸収スペクトルである。

【図5】エポキシノマイシンAの重メタノール溶液
(内部標準：トリメチルシラン)にて測定したプロトン

核磁気共鳴スペクトルである。

(内部標準：トリメチルシラン) にて測定した投薬13核
磁気共鳴スペクトルである。

【図7】エポキシノマイシンBのメタノール溶液の紫外線吸収スペクトルである。

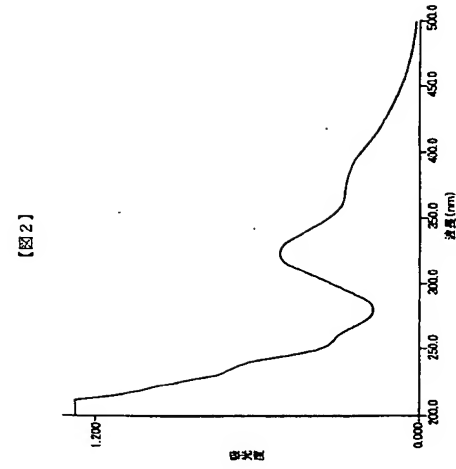
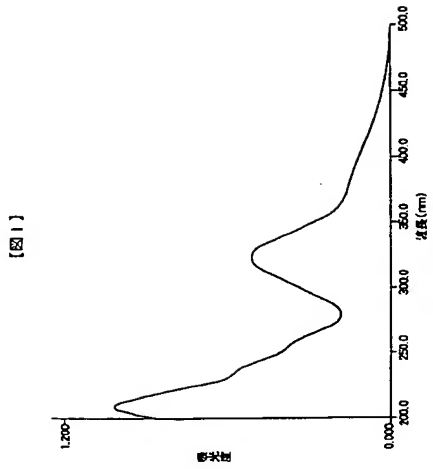
【図8】エポキシノマイシンBの0.01N NaOH-メタノール溶液中の紫外吸収スペクトルである。

【図9】エポキシノマイシンBの0.01N HCl-メタノール溶液中の紫外線吸収スペクトルである。

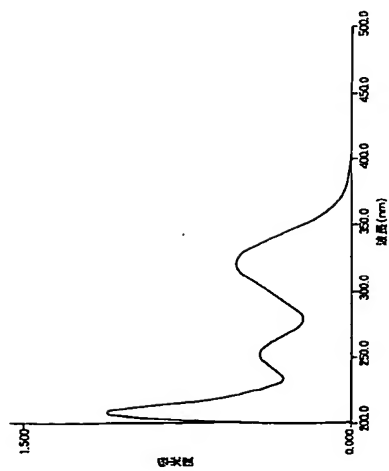
【図10】エポキシノマイシンBのKBr錠剤法で測定した赤外線吸収スペクトルである。

【図11】エポキシノマイシンBの重メタノール溶液
(内包構造：トリメチルシラン)にて測定したプロトン

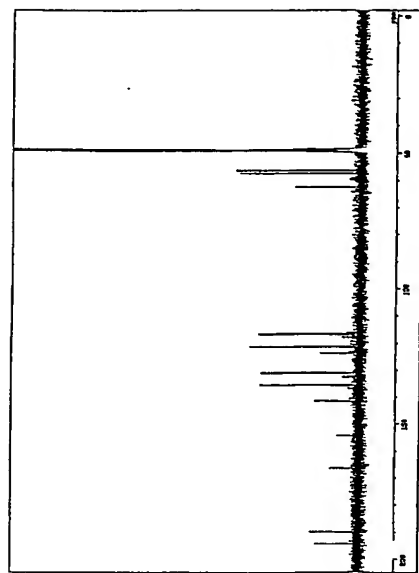
核磁気共鳴スペクトルである。



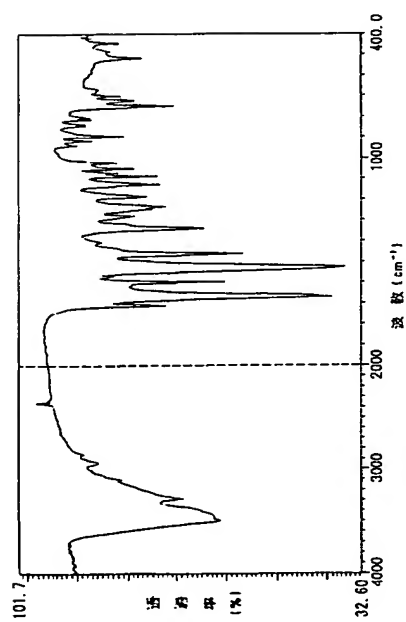
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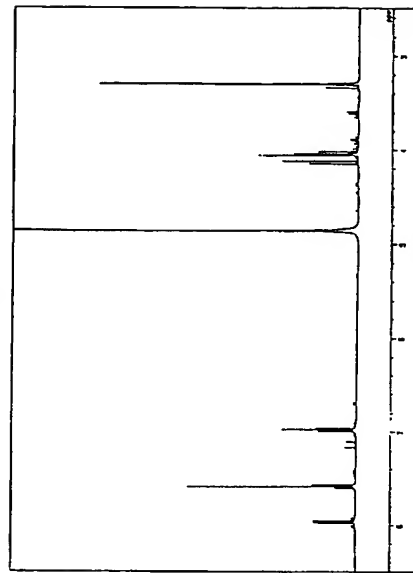
【図5】



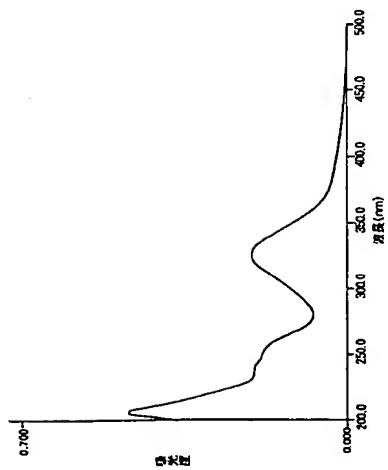
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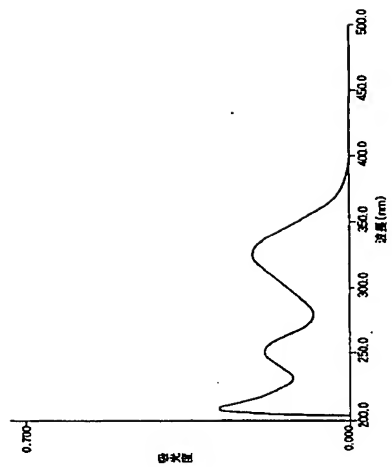
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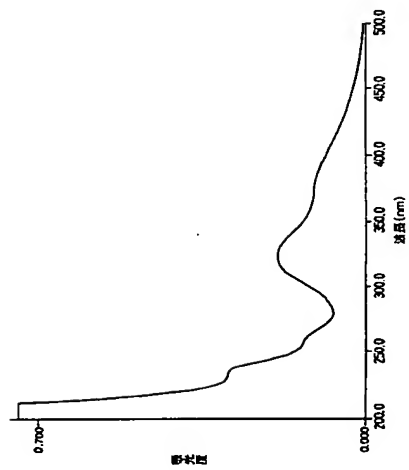
【図7】



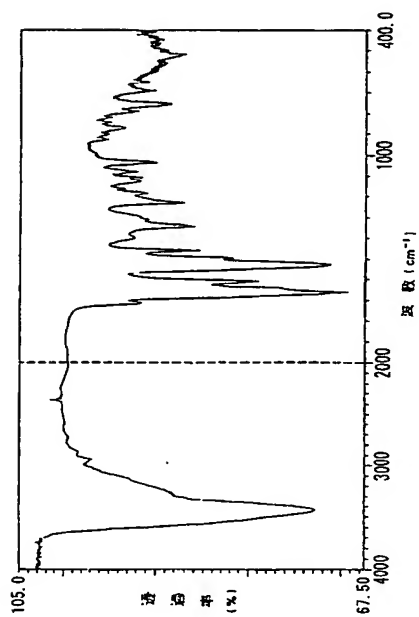
【図9】



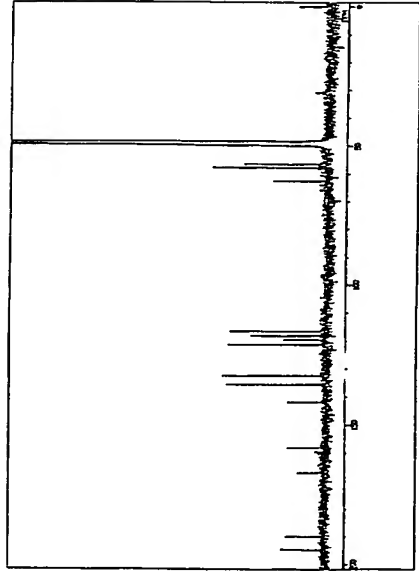
【図8】



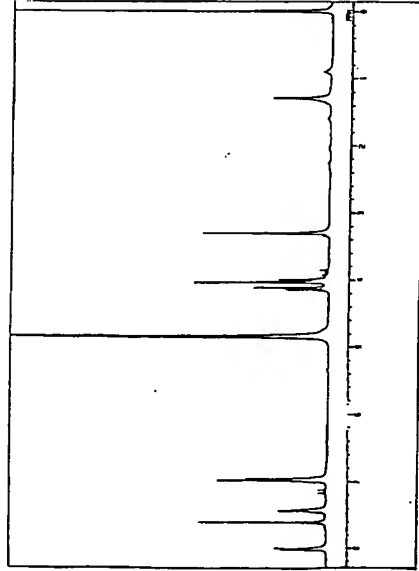
【図10】



【図 11】



【図 12】



【手続補正書】
【提出日】平成8年4月26日
【手続補正1】
【補正対象書類名】明細書
【補正対象項目名】0010
【補正方法】変更
【補正内容】
【0010】A) 抗菌活性

本発明による抗生物質エボキシノマイシンAおよびBの各種細菌に対する最低抑制濃度は、次の表1に示す通りである。この抗菌スペクトルは日本化学療法学会標準法に基づき、ミューラー-ヒントン寒天培地で倍數希釈法により測定した。
【手続補正2】
【補正対象書類名】明細書

【補正対象項目名】0021

【補正方法】変更

【補正内容】

【0021】(4) 炭素源の利用性 (ブリードハム・ゴドリープ寒天培地、ISP-培地9:27℃培養)
d-グルコース、d-フルクトース、イノシトール、d-マンニトールを利用して発育し、L-アラビノース、シュクロース、ラムノース、ラフィノースは利用しな

い、d-キシロースの利用の存否は判断しない。
(5) リンゴ酸石灰の溶解 (リンゴ酸石灰寒天培地、27℃培養)
培養後10日目よりリンゴ酸石灰の溶解が認められ、その作用は中等度である。
(6) 硝酸塩の還元反応 (0.1%硝酸カリウム含有ペプトン水、ISP-培地8、27℃培養)
陰性である。

フロントページの続き

(51) Int. Cl.⁶
C12R 1:01

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PATENT ABSTRACTS OF JAPAN

(11)Publication number : 09-157266
(43)Date of publication of application : 17.06.1997

(51)Int.Cl.

C07D303/36
A61K 31/335
A61K 31/335
C12P 17/02
//C12P 17/02
C12R 1:01)

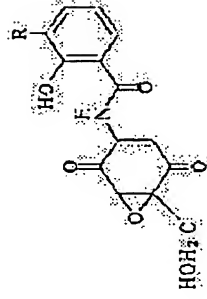
(21)Application number : 07-315542 (71)Applicant : MICROBIAL CHEM RES FOUND
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TSUCHIDA TOSHIO
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OSANAWA HIROSHI
HAMADA MASA

(54) NEW ANTIBIOTIC EPOXYNOMICIN A AND B AND THEIR PRODUCTION

(57)Abstract:

PROBLEM TO BE SOLVED: To obtain new antibiotics, epoxynomicin A and B, exhibiting antitumor activities and antibacterial activities against gram-positive bacteria including methicillin-resistant bacteria.

SOLUTION: Antibiotics, epoxynomicin A and B, of the formula (R is chlorine and H in the epoxynomicin A and B, respectively). The epoxynomicin A has the following physicochemical properties. Appearance and properties: pale yellowish powder, weakly acidic substance; melting point: 168-173° C (decomposition); specific rotation: $[\alpha]_{D25+44.6}^{\circ}$ (C 0.51, methanol); Rf value of TLC: 0.28; high resolution mass spectrum: experimental value: 332.0136 (M-H), calculated value: 332.0118; molecular formula: C₁₄H₁₀NO₆Cl; UV light spectrum: λ_{max} nm (ε): 236 (sh,8900), 255 (sh,5900), 325 (sh, 8000), 370 (sh, 2700) (methanol solution). The compound of the formula is obtained by culturing an epoxynomicin A and B-producing fungus [Amycolatopsis sp. MK 299-95F4 (FERM P-15243)] belonging to the genus Amycolatopsis in a nutritive medium under an aerobic condition.



LEGAL STATUS

[Date of request for examination]
[Date of sending the examiner's decision of rejection]
[Kind of final disposal of application other than

the examiner's decision of rejection or application converted registration]
[Date of final disposal for application]
[Patent number]
[Date of registration]
[Number of appeal against examiner's decision of rejection]
[Date of requesting appeal against examiner's decision of rejection]
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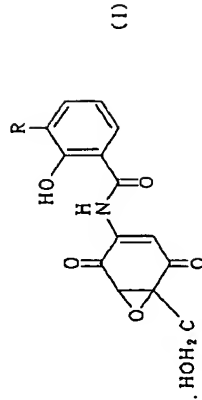
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- 3.In the drawings, any words are not translated.

CLAIMS

[Claim(s)]

[Claim 1] The following general formula (1) : HOH₂C

They are the antibiotic epoxy quinomycin A which is expressed with (R showing a chlorine atom by epoxy quinomycin A, and showing a hydrogen atom by epoxy kino mycin B among a formula) and which is a compound and epoxy kino mycin B, or those salts.

[Claim 2] the manufacturing method of the antibiotic epoxy quinomycin A which cultivates the epoxy quinomycin A according to claim 1 belonging to the Amycolatopsis group, and the production bacillus of B to a nutrition culture medium, and is characterized by extracting epoxy quinomycin A and (or) B from a culture, and (or) epoxy kino mycin B.

[Claim 3] the antimicrobial agent which makes an active principle antibiotic epoxy quinomycin A and (or) epoxy kino mycin B, or those salts.

[Claim 4] the antitumor agent which makes an active principle antibiotic epoxy quinomycin A and (or) epoxy kino mycin B, or those salts.

[Claim 5] Amycolatopsis with the property of producing antibiotic epoxy quinomycin A and epoxy kino mycin B sp.MK299-95F4 Stock.

[Translation done.]

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- 3.In the drawings, any words are not translated.

DETAILED DESCRIPTION

[Detailed Description of the Invention]

[0001] [Field of the Invention] this invention relates to the manufacturing method of epoxy quinomycin A and (or) epoxy kino mycin B, concerning the new antibiotic epoxy kino mycin (Epoxyquinomycin) A which shows antimicrobial activity and antitumor activity, or anticancer activity and epoxy kino mycin B, or these salts, furthermore, this invention relates to the antimicrobial agent and antitumor agent which make an active principle epoxy quinomycin A and (or) epoxy kino mycin B, or those salts. Moreover, this invention is Amycolatopsis as a new microorganism with the property of producing new antibiotic epoxy quinomycin A and B. sp.MK299-95F4 A stock is included.

[0002] [Description of the Prior Art] The antibacterial substance of various large number is known, and the anticancer matter of various large number is known.

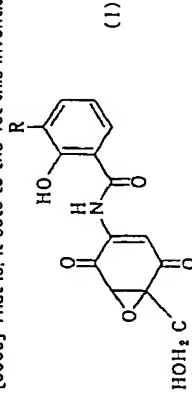
[0003]

[Problem(s) to be Solved by the Invention] In the chemotherapy of the microbism, the appearance of a drug-resistant strain is a serious problem. To carry out the discovery or the invention of a new compound whose known antibacterial compound currently used or it is known conventionally shows the antimicrobial activity which has the different chemical structure and was excellent is always desired, and research for it is done. Moreover, the anticancer matter has many which generally have strong toxicity, and serves as big constraint in the use as the antitumor agent. Then, toxicity is always wanted to discover or invent the anticancer matter which has the low and new chemical structure, and research for it is done.

[0004]

[Means for Solving the Problem] this invention persons have promoted development of an antibiotic more useful than before and research of utilization for the purpose of offering a new antibiotic with the antimicrobial activity and antitumor activity which can meet the above-mentioned request. Consequently, it found out producing two or more antibiotics which succeed in separating the strain which belongs to the Amycolatopsis group as a new microorganism from a soil sample, and have a soil skeleton with this new strain. It succeeded in isolating two sorts of these new antibiotics, and each was named epoxy quinomycin A and epoxy kino mycin B. Furthermore, it found out that antimicrobial activity was shown in the gram-positive bacteria with which these new antibiotics contain drug resistance bacteria (methicillin resistant bacteria etc.), and control activity was shown to growth of a cancer cell.

[0005] That is, it sets to the 1st this invention and is the following general formula (I) :



The epoxy quinomycin A which is the compound expressed with (R showing a chlorine atom by epoxy quinomycin A, and showing a hydrogen atom by epoxy kino mycin B among a formula) and epoxy kino mycin B, or these salts are offered.

[0006] It is the weak acidic matter, and as those salts, there is a salt with organic bases, such as quarternary ammonium salt, or a salt with various metals, for example, a salt with alkali metal like sodium, and, as for epoxy quinomycin A and B, these salts also have above-mentioned antimicrobial activity and antitumor activity.

[0007] next, antibiotic epoxy quinomycin A and B are physicochemical --- description is indicated.

(1) epoxy quinomycin A is physicochemical --- description --- A appearance and property: --- light yellow fine particles and weak acidic matter B melting point: 168 to 173 degree C (decomposition)

With the thin-layer chromatography of 0.28 silica gel (Art.105715, Merck Co. make). The Rf value of 25-44.6 degree (c 0.51, methanol) DTLC of specific-rotation:[alpha] D : C) As an expansion solvent When it developed and measures with a chloroform-methanol (10:1) E mass spectrum (m/z) : 324 326(M+H)+] 322, 324(M-H)- F high-resolution mass spectrum: Experimental value 322.0136(M-H)- calculated value 322.0118G molecular formula: --- C14H10NO6 ClH ultraviolet absorption spectrum: --- UV absorption spectrum measured in (i) methanol solution is shown in drawing 1 of an accompanying drawing. The main peaks are as follows.

lambdamax nm (epsilon)236 (sh, 8900), 255 (sh, 5900), 325 (8000), 370(sh, 2700) (ii)0.01N UV absorption spectrum measured in the NaOH-methanol solution is shown in drawing 2 of an accompanying drawing. The main peaks are as follows.

UV absorption spectrum measured in lambdamax nm (epsilon)234 (sh, 11600), 257 (sh, 5100), 327 (8300), and a 371 (sh, 4400) (iii) 0.01N HCl-methanol solution is shown in drawing 3 of an accompanying drawing. The main peaks are as follows.

lambdamax nm (epsilon)253 (6700), a 322(8500) 1 infrared absorption spectrum (KBr briquette method): It is shown in drawing 4 of an accompanying drawing.

numax (cm-1) 3450, 1710, 1670, 1600, 1520, 1460, 1340, a 1230J 13 C-NMR spectrum (CD3 OD/TMS): It is shown in drawing 5 of an accompanying drawing.

K) 1 H-NMR spectrum (CD3 OD/TMS) : it is shown in drawing 6 of an accompanying drawing.

[0008] (2) epoxy kino mycin B is physicochemical --- description --- A appearance and property: --- light yellow fine particles and weak acidic matter B melting point: 178 to 184 degree C (decomposition)

With the thin-layer chromatography of 0.52 silica gel (Art.105715, Merck Co. make). The Rf value of 25-32.2 degree (c 0.51, methanol) DTLC of specific-rotation:[alpha] D : C) As an expansion solvent When it developed and measures with a chloroform-methanol (10:1) E mass spectrum (m/z) : 289(M)+ 288(M-H)- F high-resolution mass spectrum: Experimental value 290.0856(M+H) + Calculated value 290.0664G molecular formula: --- C14H11NO6H ultraviolet absorption spectrum: --- UV absorption spectrum measured in (i) methanol solution is shown in drawing 7 of an accompanying drawing. The main peaks are as follows.

lambdamax nm (epsilon)237 (6100), 253 (sh, 5400), 326(6300) (ii)0.01N The absorption spectrum measured in the NaOH-methanol solution is shown in drawing 8 of an accompanying drawing. The main peaks are as follows.

UV spectrum measured in lambdamax nm (epsilon)235 (9100), 259 (sh, 4000), 324 (5800), and a 376 (sh, 3400) (iii) 0.01N HCl-methanol solution is shown in drawing 9 of an accompanying drawing. The main peaks are as follows.

lambdamax nm (epsilon)252 (5700), a 327(6500) 1 infrared absorption spectrum (KBr briquette method): It is shown in drawing 10 of an accompanying drawing.

numax (cm-1) 3430, 1710, 1660, 1610, 1530, 1340, a 1230J 13 C-NMR spectrum (CD3 OD/TMS): It is shown in drawing 11 of an accompanying drawing.

K) 1 H-NMR spectrum (CD3 OD/TMS) : it is shown in drawing 12 of an accompanying drawing. [0009] furthermore, antibiotic epoxy quinomycin A and B are biological --- description is indicated below.

[0010] A) The antibiotic epoxy quinomycin A by antimicrobial activity this invention and the minimum growth inhibition concentration [usually as opposed to the various bacteria on a nutrient agar plate] of B are as being shown in the next table 1. This antimicrobial spectrum was measured with the multiple dilution method by ***** and the Mueller HINTON agar medium by the Japanese Society of Chemotherapy standard method.

[0011]
(表 1)

試 験 菌	最低発育阻止濃度 (μg/ml)	
	エポキシキノ マイシン A	エポキシキノ マイシン B
スタヒロコッカス・アウレウス FMA 208P	12.5	12.5
スタヒロコッカス・アウレウス・スミス	12.5	12.5
スタヒロコッカス・アウレウス MS 3610	50	25
スタヒロコッカス・アウレウス MRSA No.5	25	25
スタヒロコッカス・アウレウス MS 16P25	25	25
スタヒロコッカス・アウレウス TY-04282	50	25
ミクロコッカス・ルチウス FMA 16	12.5	25
ミクロコッカス・ルチウス IFU 3333	3.12	6.25
バシリス・アンシラシス	25	12.5
バシリス・サブチリス NRRL B-558	50	12.5
バシリス・セレウス ATCC 10702	25	12.5
コリネバクテリウム・ボビス 1810	50	50
エシェリヒア・コリ NIJH	100	50
エシェリヒア・コリ BE 1121	50	50
エシェリヒア・コリ BB 1186	50	50
シガラ・ディセンテリエ JS 11910	50	50
シウドモナス・エルギノサ A 3	>50	>50
バストレラ・ピジシダ sp. 5395	12.5	12.5
バストレラ・ピジシダ sp. 0356	12.5	12.5
バストレラ・ピジシダ p-3347	3.12	12.5

[0012] B) The concentration (IC50 value) of the epoxy quinomycin A which controls growth of a cancer cell 50% using the cancer cell of cancer cell growth control activity various kinds, and epoxy kino mycin B was measured by the MTT method (" Journal of Immunological Methods" refer to 65 volumes, and 55 - 60 pages (1983)). The result is shown in Table 2.

[0013]

(表 2)

供 試 菌 株	IC ₅₀ (μg/ml)	
	エポキシキノ マイシン A	エポキシキノ マイシン B
マウス白血腫 L1210	2.64	16.3
マウス IMCカルシノーマ	9.67	17.8
マウスサルコーマ S180	7.67	
マウス黒色腫 B16-BL6	7.97	

[0014] Since the antibiotic epoxy quinomycin A and B by this invention have antimicrobial activity to various kinds of bacteria, they are useful as an antimicrobial agent, so that clearly from the result of Table 1. Moreover, since epoxy quinomycin A and B have the antitumor activity or anticancer activity which controls growth of various kinds of cancer cells, they are useful as an antitumor agent or an anticancer agent, so that clearly from the result of Table 2. [0015] furthermore, according to the 2nd this invention, the epoxy quinomycin A of the aforementioned general formula (I) belonging to the Amycolatopsis group and the production bacillus of B are cultivated to a nutrition culture medium, and the manufacturing method of the antibiotic epoxy quinomycin A characterized by extracting epoxy quinomycin A and (or) epoxy kino mycin B from a culture and (or) epoxy kino mycin B is offered.

[0016] As an example of the epoxy quinomycin A which can be used by the approach of the 2nd this invention, and the production bacillus of B, it is Amycolatopsis. sp.MK299-95F4 There is a stock. In a microorganism national chemical laboratory, this strain is the Actinomyces separated from the soil of Sendai, Miyagi, and will be the microorganism to which the strain number of MK299-95F4 was given in October, Heisei 6.

[0017] This MK299-95F4 share mycology-description is indicated below.

1. Branch gestalt radical viable cell yarn well, and it presents the letter of zigzag. Moreover, fragmentation is accepted. Aerial mycelia have the shape of direct, and the shape of irregular music, and are divided in the fragment of a cylindrical shape - an ellipse, or the spore's structure. The front face is smooth and magnitude is abbreviation. It is 0.4 to 0.6x1.1-1.6 microns, a whorl branch, *****, and a spore obtain and a movement sexual spore is not accepted.

[0018] 2. The color harmony manual (color harmony manual of Container Corporation of America) of the container corporation OBU United States was used for the criterion shown in [] about the publication of the growth condition color in various culture media.

(1) Sucrose and a nitrate agar medium (27-degree-C culture)

On colorless growth, white aerial mycelium is grown slightly, and soluble coloring matter is not accepted.

(2) Glucose asparagine agar medium (27-degree-C culture)

Growing white aerial mycelium on growth of light yellow [2ea, Lt Wheat-2gc, Bamboo], soluble coloring matter wears yellow.

(3) Glycerol asparagine agar medium (5 or 27 degrees-C culture of ISP-culture media)

Growing white aerial mycelium on growth of light yellow tea [3ie, Camel-3le, Cinnamon], soluble coloring matter wears yellow-brown.

(4) Starch and a mineral salt agar medium (4 or 27 degrees-C culture of ISP-culture media)

On colorless growth, white aerial mycelium is grown slightly, and soluble coloring matter is not accepted.

[0019] (5) Tyrosin agar medium (7 or 27 degrees-C culture of ISP-culture media)

Growing white aerial mycelium on growth of light yellow tea [2lg, Mustard Tan] - gray tint yellow-brown [3lg, Adobe Brown], soluble coloring matter presents light yellow tea.

(6) Nutrient agar medium (27-degree-C culture)

White aerial mycelium is slightly grown on growth of light yellow [2ea, Lt Wheat], and soluble

coloring matter is not accepted.

(7) Yeast and a malt-agar culture medium (2 or 27 degrees-C culture of ISP-culture media)
White aerial mycelium is slightly grown on growth of light yellow tea [3ic, Lt Amber], and soluble coloring matter is not accepted.

(8) Oatmeal agar medium (3 or 27 degrees-C culture of ISP-culture media)

White aerial mycelium is slightly grown on growth of colorlessness - light yellow [1 1/2ca,

Cream], and soluble coloring matter is not accepted.

(9) Starch agar medium (27-degree-C culture)

On colorless growth, white aerial mycelium is grown slightly, and soluble coloring matter is not accepted.

(10) Malic-acid lime agar medium (27-degree-C culture)

On colorless growth, white aerial mycelium is grown slightly, and soluble coloring matter is not accepted.

[0020] 3. Physiological property (1) As a result of examining using a growth temperature requirement, glucose asparagine agar medium (glucose 1.0% and L-asparagine 0.05%, potassium phosphate 0.05%, string agar 3.0%, pH7.0) at each temperature of 10 degrees C, 20 degrees C, 24 degrees C, 27 degrees C, 30 degrees C, 37 degrees C, and 50 degrees C, growth at 10 degrees C and 50 degrees C was not accepted, but be grown in 20 degrees C - 37 degrees C. Growth optimum temperature is considered to be near 27 degree C.

(2) Hydrolysis of starch (starch and a mineral salt agar medium, the ISP-culture medium 4 and a starch agar medium, and all are cultivated 27 degrees C)

In the culture for 21 days, it is negative also in which culture medium.

(3) Generation of melanin Mr. coloring matter (trypton yeast broth, ISP-culture-medium 1:peptone yeast and an iron agar medium, an ISP-culture-medium 6; thyrosin agar medium, the ISP-culture medium 7; all are cultivated 27 degrees C)

Also in which culture medium, it is negative.

[0021] (4) Availability of a carbon source (9; 27 degrees-C culture of PURIDOHAMU GODORIBU agar-medium and ISP-culture media)

It grows using D-glucose, D-fructose, an inositol, and D-mannitol, and L-arabinose, sucrose, rhamnose, and a raffinose are not used. It is not [the existence or nonexistence of use of D-xylose] ascertained.

(5) The dissolution of malic-acid lime (a malic-acid lime agar medium, 27-degree-C culture)

The dissolution of malic-acid lime is accepted around [after culture] the 10th, and the operation is whenever [middle].

(6) The reduction reaction of a nitrate (8 or 27 degrees-C culture of 0.1% potassium-nitrate content peptone water and ISP-culture media)

It is negative.

[0023] If the above description is summarized, on the gestalt, MK299-95F4 share will branch radical viable cell yarn well, will present the shape of JIGUZAKU, and will accept fragmentation. Aerial mycelia have the shape of direct, and the shape of irregular music, and are divided in the fragment of a cylindrical shape - an ellipse, or the spore's structure, a whorl branch, ***** , and a spore obtain and a movement sexual spore is not accepted. By various culture media, white aerial mycelium is grown on growth of colorlessness light yellow - light yellow tea. Soluble coloring matter wears yellow or yellow-brown by a part of culture media. Each of generation of melanin Mr. coloring matter, water solubility of starch, and reduction reactions of a nitrate is negative.

[0023] By the way, the MK299-95F4 share fungus body component showed the cell wall type IV mold to the cell wall including the 2,6-diaminopimelic acid, the arabinose, and the galactose of a meso mold. The reducing sugar in [all] a fungus body were A molds containing arabinose and a galactose. The result of a glycolate test was an acetyl mold. Moreover, mycolic acid was not contained, but phospholipid was a PII mold (phosphatidylcholine and strange glucosamine content phospholipid are not included including phosphatidylethanolamine), and main menaquinones were MK-9 (H4), a fatty acid -- 16:0, i-15:0, 16:1, and i- 16:0 and 17:0 were used as the principal component.

[0024] From the above result, MK299-95F4 share is Amycolatopsis (Amycolatopsis). It is thought that it belongs to a group (reference: "International Journal of Systematic Bacteriology" 36 volumes, 29 - 37 pages, 1986). Retrieval of the known strain of the Amycolatopsis group raised Amycolatopsis SURUFUREA (Amycolatopsis sulphurea) (reference 1:same-as-the-above; and reference 2: "International Journal of Systematic Bacteriology" 37 volumes, 292 - 295 pages, 1987) as a kind of a close relationship. Then, MK299-95F4 share and this laboratory preservation strain of Amycolatopsis SURUFUREA are [comparison] under examination to practice. This time -- MK299-95F4 share -- Amycolatopsis ESUPI (Amycolatopsis sp.) -- it is referred to as MK299-95F4. In addition, the deposition application of the MK299-95F4 share was made in National Institute of Bioscience and Human-Technology, Agency of Industrial Science and Technology, and it was entrusted with the deposition number as FERM P-15243 on October 17, Heisei 7.

[0025] In enforcing the approach of the 2nd this invention, the epoxy quinomycin A belonging to the Amycolatopsis group and the production bacillus of B are inoculated into a nutrition culture medium, and it cultivates in this culture medium. The nutrition culture medium used here contains the carbon source and nitrogen source which can carry out utilization of the aforementioned production bacillus as a nutrition component.

[0026] As the nutrient, nutrients which can be assimilated, such as what is usually used as a nutrient of a microorganism, for example, a carbon source, a nitrogen source, and mineral salt, can be used. For example, the mineral salt of dipotassium phosphate, sodium phosphate, salt, a calcium carbonate, magnesium sulfate, a manganese chloride, etc. can be used for nitrogen sources, such as the carbon source like fats and oils, such as hydrocarbons, such as grape sugar, a maltose, molasses, a dextrin, a glycerol, and starch, and soybean oil, peanut oil, and a peptone, a meat extract, cottonseed powder, a soybean meal, a yeast extract, casein, corn steep liquor, NZ-amine, an ammonium sulfate, an ammonium nitrate, and an ammonium chloride and a pan, and a trace element, for example, cobalt, iron etc. be added as occasion demands if a use bacillus can use for producing antibiotic epoxy quinomycin A and B in addition to this as a nutrient, any well-known nutrient can be used.

[0027] Especially the blending ratio of coal of the nutrient like the above in a culture medium is not restrained, can continue broadly and can be changed, and if the optimal presentation and the optimal blending ratio of coal of a nutrient are a person concerned by the epoxy quinomycin A and B production bacillus to be used, an easy bench scale test can determine easily. Moreover, the nutrition culture medium which consists of the above-mentioned nutrient can be sterilized in advance of culture, and, as for the front stirrup of this sterilization, it is advantageous to adjust pH of a culture medium later in the range of 6-8, especially the range of pH 6.5-7.5.

[0028] Culture of the epoxy quinomycin A in this nutrition culture medium and B production bacillus can be performed according to the approach usually used in manufacture of the antibiotic by the common Actinomycetes. Usually, it can carry [while cultivating under an aerobic condition is suitable and it stirs and/or] out, carrying out aeration. Moreover, although both stationary culture shaking culture and the submerged culture accompanied by aeration stirring are usable as the culture approach, liquid culture is suitable for mass production method of epoxy quinomycin A and B.

[0029] Although the culture temperature which can be used can be suitably chosen according to the production bacillus which growth of epoxy quinomycin A and B production bacillus is not substantially checked, it is not especially restricted if it is the range which can produce this antibiotic, and is used, especially a desirable thing can mention the temperature within the limits of 25 to 30 degree C. Culture is continuable until epoxy quinomycin A and B are usually fully accumulated. Although the culture time amount changes with the presentation of a culture medium, culture temperature and service temperature, use production strain, etc., the target antibiotic can usually be obtained by culture of 72-120 hours.

[0030] The epoxy quinomycin A in the culture medium under culture and the accumulated dose of B can use staphylococcus AUREUSU Smith, and he can do a quantum with the cup method used for the quantum of the usual antibiotic.

[0031] The epoxy quinomycin A and B which were accumulated into the culture in this way

extract this from a culture. After culture and according to the need, after removing a fungus body from a culture by the separation approaches well-known in itself, such as filtration and centrifugal separation, isolation purification of the chromatography using the chromatography and gel filtration which adjusted the culture filtrate to acidity (pH 2-4), and used the solvent extraction using an organic solvent, especially ethyl acetate, etc., adsorption, and ion-exchange ability, and countercurrent distribution can be carried out by using it, being independent or combining, and the target antibiotic can be extracted. As support for chromatographies which has adsorption and ion-exchange ability, activated carbon, silica gel, porous polystyrene-divinylbenzene resin, or various kinds of ion exchange resin can be used. Moreover, from the separated fungus body, the target antibiotic can be extracted from a fungus body by the solvent extraction method using a suitable organic solvent, or the melting by fungus body crushing, and isolation purification can be carried out like the above. The new antibiotic epoxy quinomycin A and B which have the above mentioned property in this way are obtained.

[00032] furthermore, in the 3rd this invention, the antimicrobial agent which makes an active principle the epoxy quinomycin A expressed with a general formula (I) and (or) epoxy kino mycin B, or those pharmaceutically permissible salts is offered.

[00033] moreover, in the 4th this invention, the antitumor agent which makes an active principle the epoxy quinomycin A expressed with a general formula (I) and (or) epoxy kino mycin B, or those pharmaceutically permissible salts is offered.

[00034] in this antimicrobial agent or antitumor agent, the epoxy quinomycin A as an active principle and (or) B, or its salt can be a formal constituent with which it is mixed with the solid-state of pharmaceutically permissible daily use or liquid support, for example, ethanol, water, starch, etc.

[00035] Moreover, Amycolatopsis with the property of producing the epoxy quinomycin A of the aforementioned general formula (I), and B as a new microorganism in the 5th this invention sp.MK299-95F4 A stock is offered.

[00036]

[Embodiment of the Invention] Next, although an example explains this invention to a detail further, this invention is not limited to the following example.

[00037] Example 1 Antibiotic epoxy quinomycin A and manufacture glycerol of B 0.5%, shoe cloth 2%, soybean meal 1%, dry yeast 1%, corn steep liquor 0.5%, cobalt chloride Liquid medium containing 0.001% (it adjusts to pH7.0) Erlenmeyer flask (500ml **) It pours 110ml distributively at a time, and is a conventional method. It sterilized at 120 degrees C for 20 minutes.

Amycolatopsis cultivated to these culture media at the agar slant medium sp.MK299-95F4 The stock (FERM P-15243) was inoculated and rotary shaking culture was carried out for five days at 30 degrees C after that. This obtained *** culture medium.

[00038] Glycerol 2%, dextrin 2%, bacto-SOITON 1%, powder yeast extract 0.3%, ammonium sulfate 0.2%, calcium carbonate Liquid medium which contains one drop of silicone oil 0.2% (it adjusts to pH7.4) Erlenmeyer flask (500ml **) It pours 110ml distributively at a time, and is a conventional method. It sterilized at 120 degrees C for 20 minutes. Then, it inoculated the 2ml of the above-mentioned *** culture medium into these culture media at a time, respectively, and rotary shaking culture was carried out to them for four days at 27 degrees C.

[00039] Thus, the obtained culture medium was filtered and the fungus body was separated. 2.55l. of culture filtrates is 6 N-HCl. After making it pH2, it extracted by 2.55l. of butyl acetate, and the butyl-acetate layer was dried with anhydrous sodium sulfate. Concentration hardening by drying was carried out under reduced pressure of a butyl-acetate layer, residue was melted to methanol 50ml, it washed twice by hexane 50ml, and concentration hardening by drying was carried out under reduced pressure of a methanol layer.

[00040] If chloroform-methanol-water (50:10:40,100ml) distributes the obtained residue and concentration hardening by drying is carried out under reduced pressure of a lower layer, it is brown oily matter (0.515g). It was obtained. This oily matter was given to the silica gel column chromatography (Kieselgel 60, the Merck Co. make, 50ml), and sequential elution was carried out with the toluene-acetone mixed solvent (1 three: 10:1, 7:1, 5:1, 2:1). The obtained activity fraction was given to the silica gel column chromatography of these conditions, and sequential

elution was carried out with the toluene-acetone mixed solvent (1 ten: 50:1, 20:1, 7:1). Epoxy quinomycin A and the mixture of B 124mg was obtained. Separation purification was carried out having bet 35mg of this mixture on silica gel TLC (expansion solvent: a chloroform-methanol, 20:1).

[0041] Epoxy quinomycin A is the melting point. It is obtained with the yield of 20mg as light yellow powder of 168 to 173 degree C (decomposition), and epoxy kino mycin B is the melting point. It was obtained with the yield of 10mg as light yellow powder of 178 to 184 degree C (decomposition).

[Translation done.]

* NOTICES *

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- 1.This document has been translated by computer. So the translation may not reflect the original precisely.
- 2.*** shows the word which can not be translated.
- 3.In the drawings, any words are not translated.

DESCRIPTION OF DRAWINGS

[Brief Description of the Drawings]

- [Drawing 1] It is an ultraviolet absorption spectrum in the methanol solution of epoxy quinomycin A.
- [Drawing 2] 0.01Ns of epoxy quinomycin A It is an ultraviolet absorption spectrum in a NaOH-methanol solution.
- [Drawing 3] It is an ultraviolet absorption spectrum in the 0.01N HCl-methanol solution of epoxy quinomycin A.
- [Drawing 4] It is the infrared absorption spectrum measured with the KBr briquette method of epoxy quinomycin A.
- [Drawing 5] It is the proton nuclear-magnetic-resonance spectrum measured with the heavy methanol solution (internal standard: trimethyl silane) of epoxy quinomycin A.
- [Drawing 6] It is the carbon 13 nuclear-magnetic-resonance spectrum measured with the heavy methanol solution (internal standard: trimethyl silane) of epoxy quinomycin A.
- [Drawing 7] It is the ultraviolet absorption spectrum of the methanol solution of epoxy kino mycin B.
- [Drawing 8] 0.01Ns of epoxy kino mycin B It is an ultraviolet absorption spectrum in a NaOH-methanol solution.
- [Drawing 9] It is an ultraviolet absorption spectrum in the 0.01N HCl-methanol solution of epoxy kino mycin B.
- [Drawing 10] It is the infrared absorption spectrum measured with the KBr briquette method of epoxy kino mycin B.
- [Drawing 11] It is the proton nuclear-magnetic-resonance spectrum measured with the heavy methanol solution (internal standard: trimethyl silane) of epoxy kino mycin B.
- [Drawing 12] It is the carbon 13 nuclear-magnetic-resonance spectrum measured with the heavy methanol solution (internal standard: trimethyl silane) of epoxy kino mycin B.

[Translation done.]